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FINE STRUCTURE OF MUSCULATURE IN THE COPEPOD *PARANTHESSIUS ANEMONIAE* CLAUS

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Paranthessius anemoniae Claus is a cyclopoid associate of the snakelocks anemone *Anemonia sulcata* (Pennant). First described by Claus (1889) in the Adriatic Sea and later by Bocquet and Stock (1959) from Mediterranean waters, *Paranthessius* has only recently been recorded from British waters (Gotto and Briggs, 1972; Briggs and Gotto, 1973; Briggs, 1973). Other recent studies of *Paranthessius* have described this copepod's general ecology (Briggs, 1976), alimentary canal (Briggs, 1977a), larval development (Briggs, 1977b) and integument (Briggs, 1978). Copepod muscle has been investigated by Hartog (1888), Scott (1901), Lowe (1935), Changeux (1960), Fahrenback (1962) and Park (1966). Ultrastructural studies of *Cyclops* by Bouligand (1962, 1963 and 1964) and of *Macrocyclops albidus* by Fahrenbach (1963) are among the few detailed studies of copepod muscle.

Materials and Methods

Copepods were fixed for 12 hr at 4° C in 5% gluteraldhyde in 0.12 M Millonig buffer (pH 7.4) containing 3% NaCl and 0.1 mM CaCl. Fixed specimens were processed for light and electron microscopy.

Light microscopy

Copepods fixed in gluteraldehyde were dehydrated though ethylene glycol and embedded in glycol methacrylate (G.M.A.) which was polymerized in gelatin capsules at 60° C for 48 hrs. Sections 1 to 2 μ thick were cut on a Reichert OMU2 ultratome and stained on glass slides with mercuric bromo phenol blue (method of Maiza, Brewer, and Alfert, 1953).

Electron microscopy

Fixed copepods were washed in Millonig buffer-wash (2–8 hrs), post-fixed for 2 hrs in 1% osmium tetroxide and the dehydrated through ethanol to propylene oxide and embedded in araldite. Sections were cut with a Reichert OMU2 ultratome, mounted on copper grids, stained with uranyl acetate and lead citrate and examined in an AE1 EM801 electron microscope operating at 60 kV.

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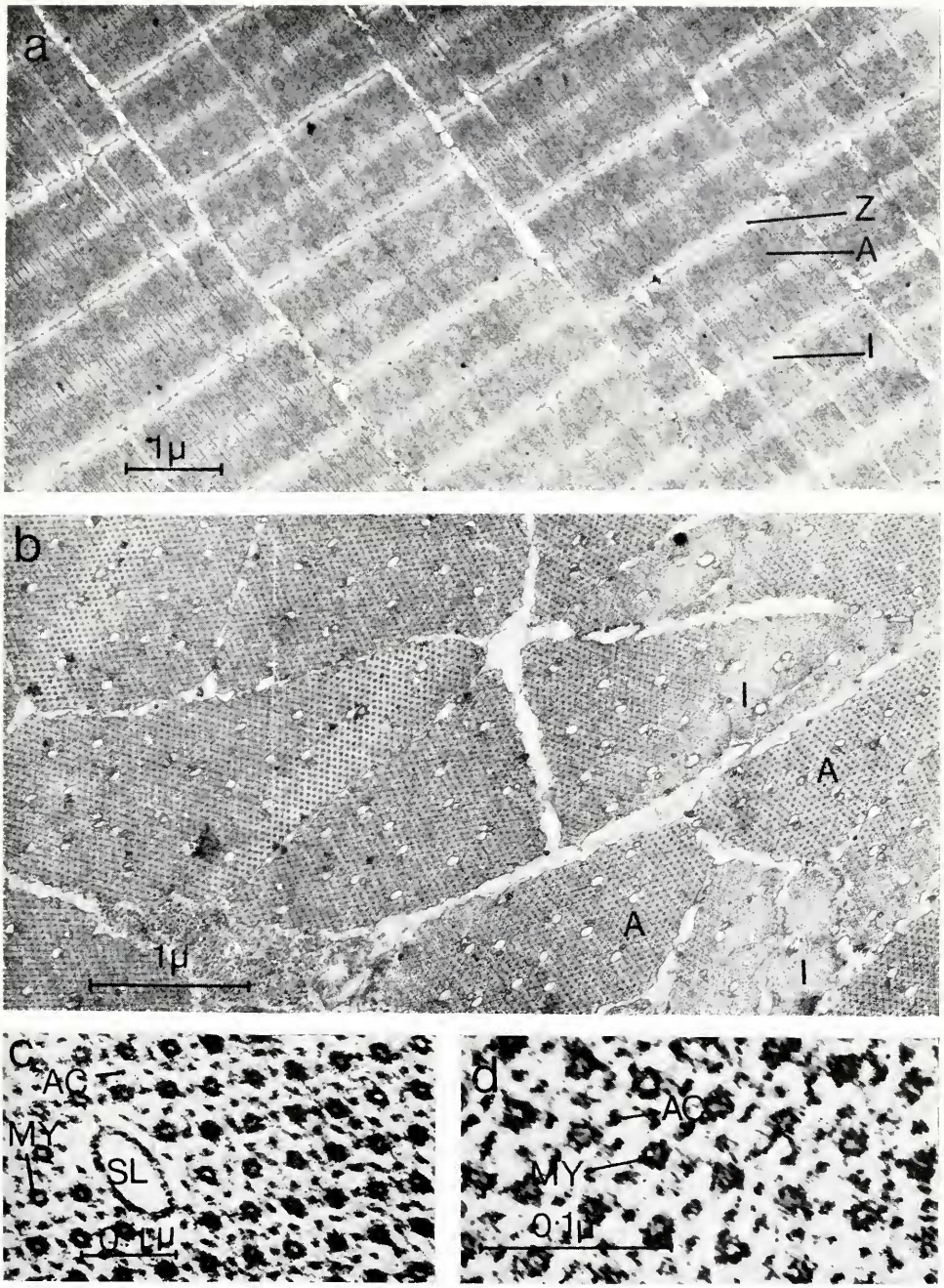

FIGURE 1. Electron micrograph of striated muscle in *Paranthessius anemoniae*. (a) Longitudinal section through muscle. Z line, A and I band are arrowed. (b) Transverse section of muscle fibrils at both A band and I band levels. (c) Transverse section of muscle showing longitudinal sarcoplasmic reticulum element (SL) actin filaments (AC) and myosin. (d) actin (AC) and myosin filaments (MY).

Results

The general body muscle

Light microscopy has shown the longitudinal muscles in *Paranthessius* to be composed of bundles of muscle fibers which pass along either side of the mid-dorsal line. A similar pair of longitudinal muscle bundles are situated in a ventro-lateral position. Treatment with periodic-acid–Schiff (method of McManus, 1946) demonstrated the presence of large amounts of glycogen within the muscle.

Examination of ultra thin sections with the electron microscope shows the muscle of *Paranthesssius* to be striated (Fig. 1a). A and I bands are clearly visible, the former having a well-defined central H zone as is usual for striated muscle (Hanson and Huxley, 1953). The functional units of muscle (sarcomeres) are separated from one another by an electron-dense Z line. Transverse section shows the muscle to be composed of polygonal-shaped myofibrils measuring between 1 and 4 μ in diameter (Fig. 1b). Both thick (myosin) and thin (actin) filaments are present. The myosin filaments, which appear to be hollow, measure 12 nm in thickness with an average length of 1100 nm and are spaced about 25 nm apart. The actin filaments, on the other hand, are on average 4 nm in thickness and are each placed equidistant between two myosin filaments. The sarcomere has an average length of 1200 nm, though this varies with the state of muscle contraction.

Superficial examination of *Paranthessius* muscle reveals an apparent similarity to vertebrate muscle with each myosin filament surrounded by six similar filaments and six actin filaments in hexagonal array (Figs. 1c, d). More detailed examination, however, shows that the actual position of the actin filaments in relation to the myosin filaments is different from vertebrate muscle in that each actin filament does not lie equidistant from three myosin filaments (Fig. 2). If the myosin filaments in *Paranthessius* are imagined to be the apices of an equilateral triangle, then the actin filaments occur in the center of each side (Fig. 2). This arrangement of myofilaments is similar to that described for other copepods, for example Bouligand (1962), Fahrenbach (1963) and Raymont, Krishnaswamy, Woodhouse, and Griffin (1974).

Sections through the muscle show the myofibrils to be surrounded by membranous material which constitutes the sarcoplasmic reticulum. This is also seen to be regularly distributed within the myofibrils. In transverse section, the interfibrillar sarcoplasmic reticulum appears as circular membranous zones, often paired, measuring 50 nm in diameter. The single or paired units are regularly spaced between 200 nm and 400 nm apart (Fig 3a). Longitudinal sections show the sarcoplasmic reticulum to be in the form of canals measuring an average of 50 nm across and of varying length (Fig. 3b), and appearing elliptical in slightly oblique sections (Fig. 3c).

Examination of a large number of muscle sections has revealed that the sarcoplasmic reticulum is a branching tubular system that runs longitudinally through and around the myofibrils. The intrafibrillar elements link up with those surrounding the fibril in the region of the Z line by means of transverse tubules of sarcoplasmic reticulum. This feature is evident in both transverse and longitudinal sections (Fig. 3d, 4a). The canals surrounding the myofibrils are continuous with the membrane of the sarcolemma surrounding the muscles. This implies the

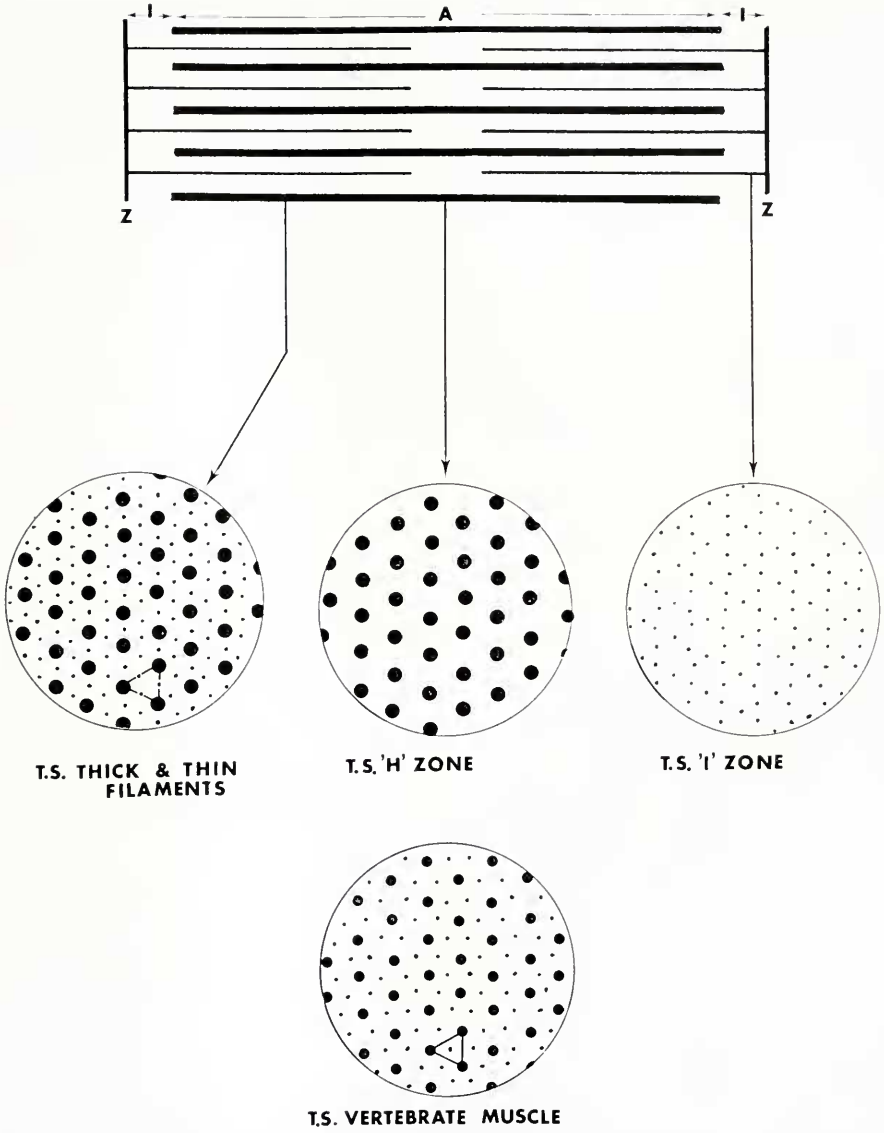

FIGURE 2. Diagrammatic representation of the myofilament arrangement in copepod muscle. Vertebrate muscle is also represented for comparison. If the myosin filaments in copepod muscle are imagined to be the apices of an equilateral triangle, then the actin filaments occur in the center of each side. In vertebrate muscle each actin filament lies equidistant from three myosin filaments.

existence of a continuous system of sarcoplasmic reticulum, leading from the muscle surface and running between the muscle fibers, myofibrils and myofilaments.

The paired nature of some of the longitudinal tubules wthin the myofibrils seen in transverse section is attributed to elongate membranous vesicles that lie along

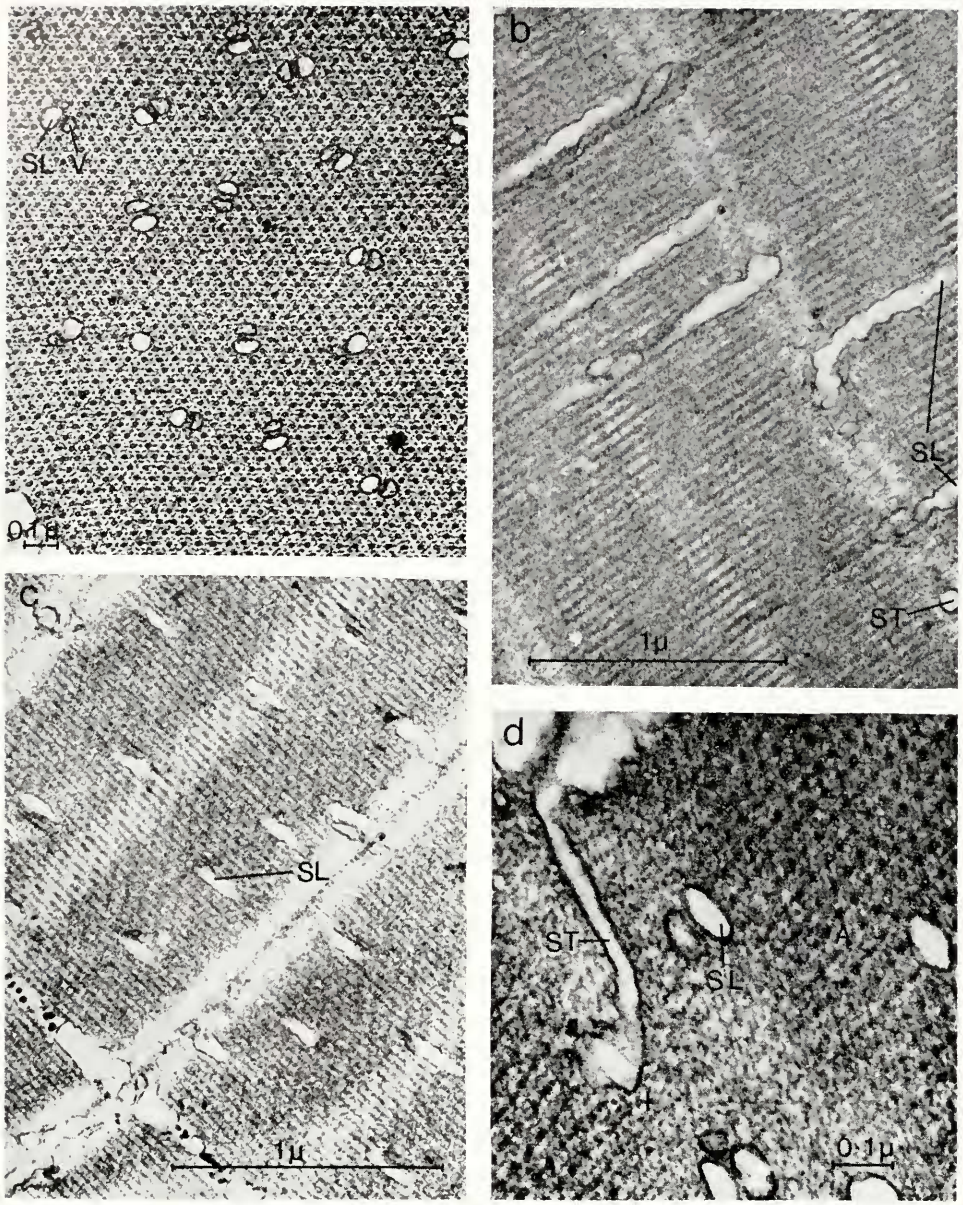

FIGURE 3. (a) Longitudinal sarcoplasmic reticulum elements (SL) and blind ending vesicles (V) in transverse section. The blind ending vesicles have a characteristic granular appearance to their lumen. (b) Longitudinal (SL) and transverse elements (ST) of sarcoplasmic reticulum as seen in longitudinal section. (c) Slightly oblique section through muscle showing longitudinal sarcoplasmic reticulum (SL) elements as oval vesicles. (d) Transverse section of *Paranthessius* muscle showing both longitudinal (SL) and transverse elements (ST) of sarcoplasmic reticulum in the I band (I). No transverse elements were observed in the A band (A).

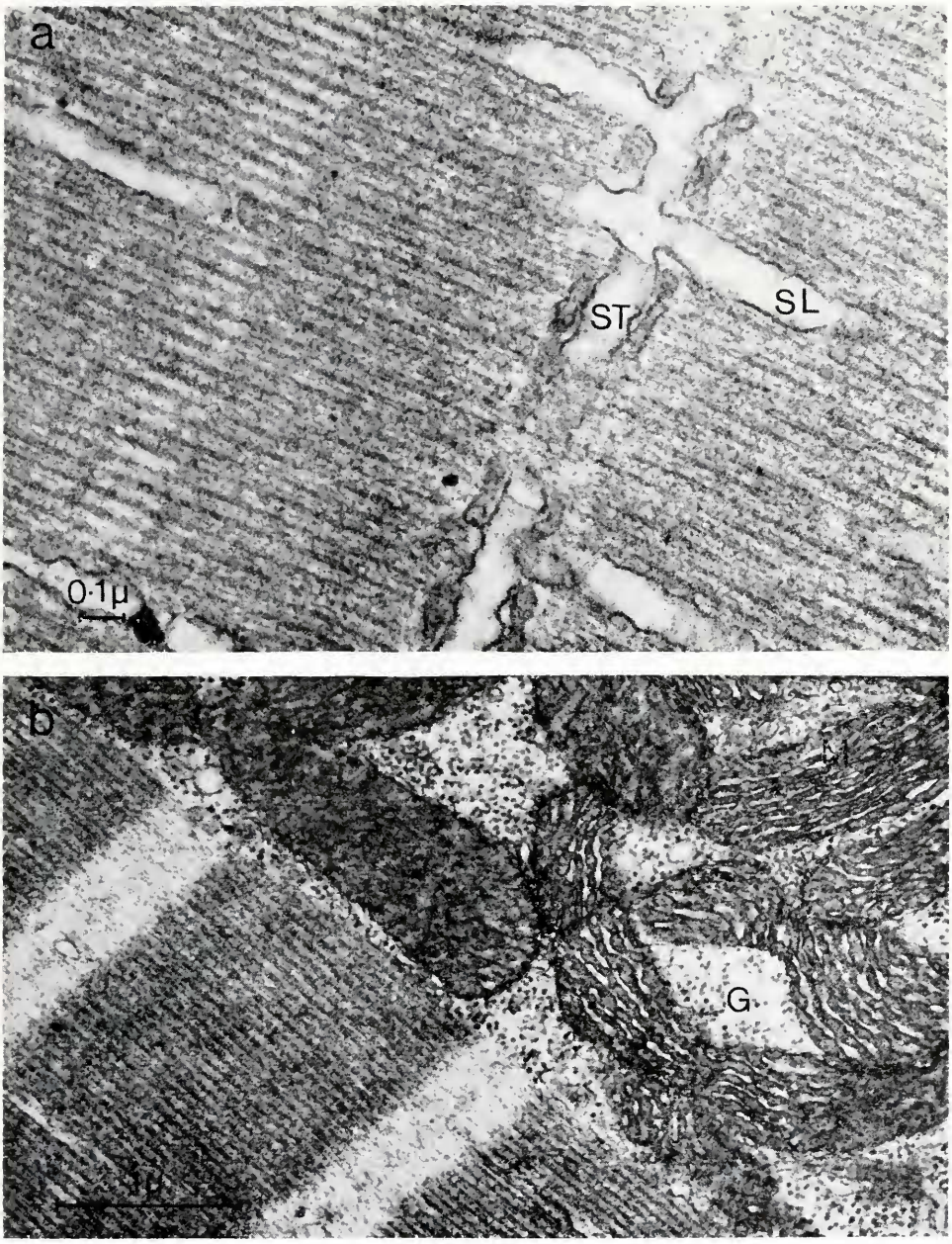

FIGURE 4. (a) Longitudinal section of *Paranthessius* muscle fibril showing junction of transverse sarcoplasmic reticulum (ST) and longitudinal sarcoplasmic reticulum (SL). (b) Mitochondria (M) and glycogen granules (G) on surface of muscle fibril.

longitudinal elements. The lumina of these vesicles have a granular appearance and their length rarely exceeds that of a sarcomere (Fig. 3a, 5). The general irregular nature and the occasional folding and branching of the longitudinal tubules

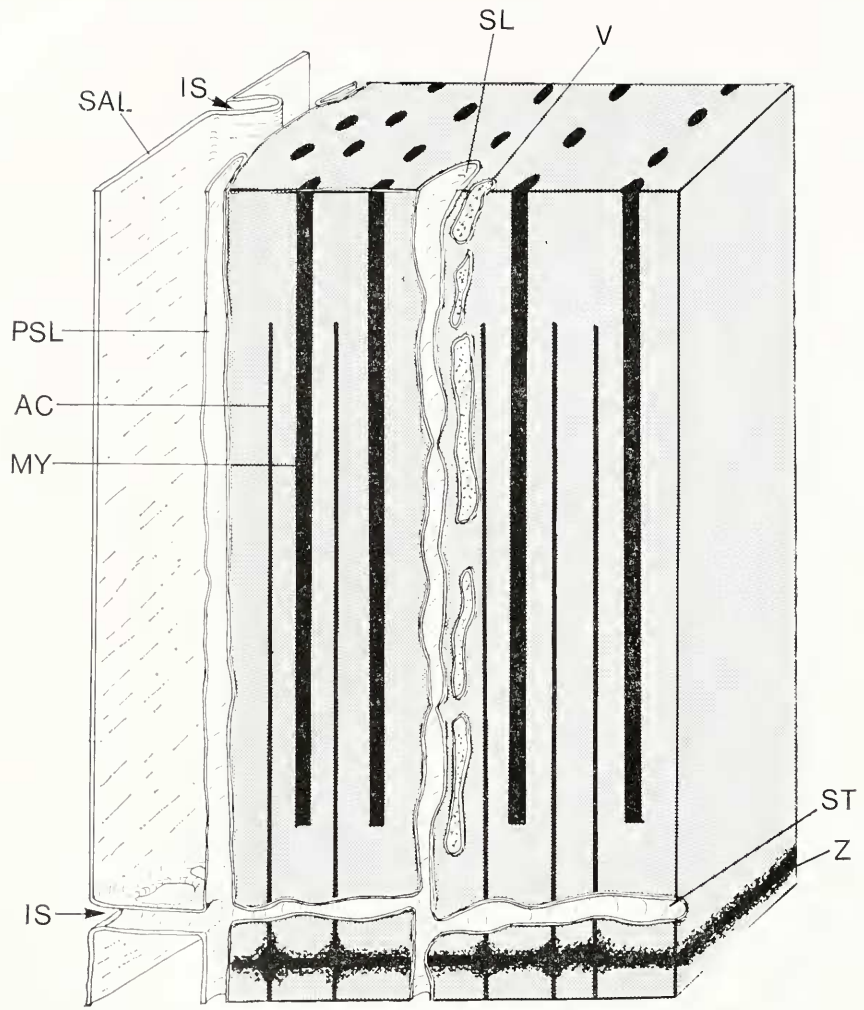

FIGURE 5. Three-dimensional diagram of the sarcoplasmic reticulum system in *Paranthessius anemoniae* muscle showing longitudinal sarcoplasmic reticulum (SL), vesicles (V), peripheral sarcoplasmic reticulum (PSL), transverse sarcoplasmic reticulum (ST), Z line (Z), actin filament (AC), myosin filament (MY), sarcolemma (SAL) and its invaginations (IS).

is probably accounted for by the occurrence of more than two units in some sections examined.

Scattered on the muscle surface under the sarcolemma are numerous mitochondria that may measure up to 2 μ in length, and 0.6 μ in width. The mitochondria of the muscle (or sarcosomes) are characterised by the possession of numerous cristae (Fig. 4b). Granules of glycogen (Fig. 4b) measuring about 25 nm in diameter occur commonly between the myofibrils and are thought to be responsible for the strong positive reaction to the P.A.S. test for carbohydrates

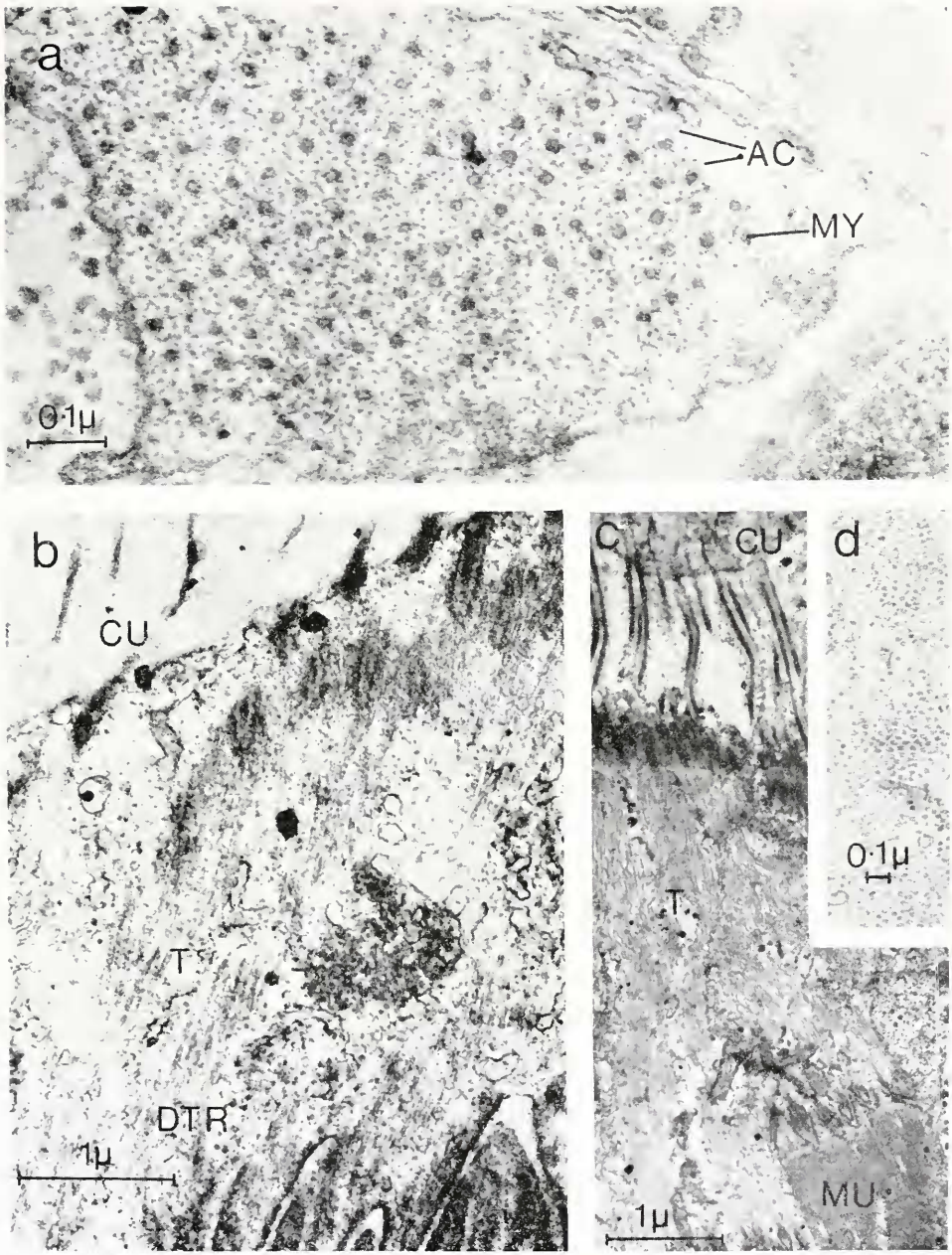

FIGURE 6. (a) Transverse section through muscle of alimentary canal showing both actin (AC) and myosin (MY) filaments. (b) Details of muscle junction with cuticle (CU) showing attachment fibrils (T) and dense terminal region (DTR) of muscle. (c) Further details of attachment of muscle (MU) to cuticle (CU) by tonofibrils (T). (d) Transverse section of the tonofibrils which attach muscle to cuticle in *Paranthessius*.

TABLE I

Dimensions of muscle fibrils in Paranthessius, Cyclops, Macrocyclops *and a vertebrate.*

Species	Myosin			Actin thickness	Reference
	length	thickness	separation		
Paranthessius	1100 nm	12 nm	25 nm	4 nm	
Cyclops	1500 nm	12 nm	25 nm	4 nm	Bouligand (1964)
Macrocyclops	—	15 nm	48 nm	—	Farenbach (1963)
Vertebrate	1500 nm	10 nm	45 nm	6 nm	Threadgold (1967)

observed in light microscope studies. The muscle nuclei are a flattened ovoid shape and have a single nucleolus. They are found in close contact with the muscle surface and were not commonly encountered during these investigations. The longitudinal muscle bundles and those supplying the appendages are constructed as described.

The muscle of the alimentary canal

The alimentary canal was found to be surrounded by muscle of a slightly different structure. There are several isolated bundles or strands of longitudinal muscle measuring 0.5 to 1.0 μ thick lying beneath the basement membrane of the digestive tract epithelium (Fig. 6a). Surrounding these longitudinal muscles is a layer of circular muscle of 1 μ average thickness. The myosin filaments of the gut muscle were seen in transverse section to be surrounded by 10 to 12 actin filaments instead of the six noted in the general body muscle. Although the myosin filaments are about the same distance apart as the general muscle (25 nm) they are somewhat thicker (15 nm). No internal ramification of sarcoplasmic reticulum is seen in this muscle.

The attachment of the muscle to the cuticle

Near the site of attachment to the cuticle the muscle fibrils terminate at an irregular electron-dense line that can be seen in longitudinal sections to traverse the fibril (Fig. 6b). More detailed examination, however, reveals that this electron-dense line is composed of the sarcolemma of the muscle, near to which, at a distance of 30 to 40 nm, is another plasma membrane that gives rise to fine tubules or tonofibrils (Fig. 6c, d). The tonofibrils measure about 20 nm in diameter and seem to pass, in groups, through thickened electron-dense zones, from each of which emerges an electron-dense fiber of roughly 400 nm thickness. These fibers ramify through the cuticle forming a firm attachment. The length of the tonofibrils ranges from 5 to 0.5 μ in different parts of the animal. Attachment is sometimes made to normal cuticle, while in other places the endocuticle invaginates to form an apophysis for muscle attachment (Briggs, 1978).

Discussion

The observations made on *Paranthessius* muscle agree in many respects with those made by Bouligand (1962, 1963, 1964) on *Cyclops* and *Acanthocyclops*, by Fahrenbach (1963) on *Macrocyclops* and by Raymont *et al.* (1974) on *Calanus*. In *Cyclops* the myosin filaments measure 1.5 μ in length compared to 1.1 μ in *Paranthessius*. *Macrocyclops* has myosin filaments 15 mm thick, which are further apart than those of both *Cyclops* and *Paranthessius*. No detailed measurements are available for *Calanus* muscle. Dimensions of muscle structures given in Table I includes details of typical vertebrate muscle for comparison.

In a study of *Acanthocyclops*, Bouligand (1964) describes zones of double overlapping between the two sets of actin filaments of the sarcomeres when the muscle is contracted. A transverse section through this zone shows twice the number of actin filaments found in other regions of the muscle. In longitudinal sections of contracted muscle these overlapping zones or CM bands are characterised by appearing as a dark band across the muscle in the center of the H zone Examination of many sections of *Paranthessius* muscle did not show this feature to be present. It is possible that the muscle examined here was fixed in the relaxed state. This is considered unlikely, however, since copepods always exhibited strong locomotory movements on encountering fixatives. The probability that all sections cut were of relaxed muscle is, therefore, very low. The general arrangement of the myofibrils is the same as that described for other copepods.

The sarcoplasmic reticulum in *Paranthessius* is very similar to that described by Bouligand (1962, 1963) for *Cyclops*, though it tends to be somewhat less elaborate than that studied by Fahrenback (1962) in *Macrocyclops*. In this species, the blind-ending membranous vesicles found in *Paranthessius* and also in *Cyclops* are more expanded, forming a well developed system of cisternae. As was found with the vesicles of *Paranthessius* muscle, the cisternae do not join with the elements of the sarcoplasmic reticulum, but come to within 10 nm in most regions of the muscle. The term dyad, used by Smith (1961), to describe the association between sarcoplasmic reticulum tubules and the cisternae in the beetle *Tenebrio* is used here to describe similar structures in copepod muscle. In *Paranthessius* a dyad represents the paired membranous tubules seen in a transverse section of muscle myofibrils. This is an association between a sarcoplasmic reticulum tubule and a blind ending vesicle.

The sarcoplasmic reticulum system of copepods may be contrasted with that of vertebrate muscle. In most vertebrates the sarcoplasmic reticulum forms a sleeve around the muscle fibril ending in a number of finger-like projections in the region of the Z line, where it comes into close contact with the invaginated membrane of the sarcolemma. This association forms a triad, composed of two sarcoplasmic reticulum elements (one from each side of the Z line of the fibril) and the membrane of the sarcolemma. The sarcolemma membrane is, therefore, not a continuous tubular system ramifying longitudinally through the myofibrils as is found in copepod muscle.

Fahrenbach (1963) stressed the importance of efficient diffusion of "transmitter substances" (Ca^{++}) in fast acting muscles. Slower contracting vertebrate muscles have the discontinuous triad structure described, in which the sarcolemma membrane

is not connected to the sarcoplasmic reticulum system. The myofilaments in the center of the myofibrils are not brought into such close proximity with a potential impulse-conducting element as is found in the arthropodan continuous dyad system of fast contracting muscle. In most fast muscle studied the distance that calcium ions have to diffuse in order to reach the center of the myofibrils to trigger construction is maintained at a minimum distance of less than 1 μ. Examples include 0.3 to 0.35 μ in the fast muscle of the dragon fly *Aeshna* (Smith, 1961), 0.18 to 0.2 μ in the toadfish *Opsanus* (Fawcett and Revel, 1961), 0.15 to 0.25 μ in the bat *Eptesicus* (Revel, 1962) and 0.2 μ in the copepod *Macrocyclops* (Fahrenbach 1963). The value for *Paranthessius* was found to be on average 0.25 μ. Fahrenbach (1963) proposes that this is the reason why the longitudinal tubules are arranged in a regular hexagonal manner in fast copepod muscle.

Bouligand (1962) suggests that the longitudinal sarcoplasmic reticulum elements of *Cyclops* are regularly arranged, so that their tendency to expand when the muscle contracts, (due to the hydrostatic pressure of their contained fluid) will not disorientate the myofilaments. Bouligand proposes that evidence for this may be gained from observation of true transverse sections of hexagonal array of the tubules. It is visualised that expansion force lines from these tubules would pass through the myosin filaments towards another expanding tubule, which would be exerting a similar force. This implies that the position of the myosin filaments would be undisturbed during muscle contraction. If the expansion forces tended to act between the myosin filaments the latter would be displaced to either side.

Both Fahrenbach's and Bouligand's interpretation of the regular arrangement of sarcoplasmic reticulum are applicable to *Paranthessius* muscle, which has a similar structure to that of the species studied by these authors. Although the sarcoplasmic reticulum is not so complex as that of *Macrocyclops*, all other structural features indicate that the muscle of *Paranthessius* is of a fast contracting type. The muscles of the alimentary canal are probably slower acting, since they have nearly twice as many actin filaments as the general body muscles which is a characteristic of "slower" muscle (Fahrenbach 1967).

Parasitic forms usually have smooth or "slow contracting muscle" (Capart, 1948). It is noteworthy that *Paranthessius* has muscle characteristics of free-living forms, *i.e.*, "fast acting muscle". This is not surprising when it is considered that *Paranthessius* is quite a mobile associated form (Briggs, 1974). It is of survival value for *Paranthessuis* to be capable of rapid swimming in order to regain a position on its host if dislodged. Since the larval instars of *Paranthessius* live freely in the plankton, the infective stage must possess efficient locomotion for host location. The elaborate musculature of the adult may, therefore, represent a legacy from the free living phase of the life cycle.

Apart from the gripping claws of the second antenna and spinal reduction in the mouth parts, *Paranthessius* is relatively unmodified morphologically and bears a strong resemblance to free-living cyclopoids. It is, moreover, associated with the external surface of the host, never being found in the gastrovascular cavity, and is very mobile both on and off the anemone. These features together with the elaborate musculature described here suggest that in an evolutionary context *Paranthessius* is a recent invader of *Anemonia sulcata*. Comparative studies of the musculature in other associated species which exhibit varying degrees of host

dependence and morphological modification might add support to these speculations on the evolution of parasitism in copepods.

I wish to thank Professor Gareth Owen and Dr. R. V. Gotto of the Queen's University of Belfast for their invaluable encouragement and advice during this study, undertaken during the tenure of a postgraduate studentship from the Department of Education for Northern Ireland.

Summary

1. *Paranthessius anemoniae* has striated muscle composed of actin and myosin myofilaments arranged hexagonally, as in free living copepods.

2. The sarcoplasmic reticulum is continuous with the membrane of the sarcolemma in the region of the Z line and forms a continuous system of tubules which ramify through the muscle.

3. Blind-ending vesicles form dyads with the longitudinal sarcoplasmic reticulum tubules.

4. Attachment of the muscle to the cuticle is by tonofibrils.

5. A relatively short sarcomere length, complex sarcoplasmic reticulum and high proportion of myosin to actin filaments indicate the "phasic" nature of the general body muscle in *Paranthessius*.

6. The muscle of the alimentary canal is characteristic of "tonic" muscle.

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